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Effect of Diet Type and Enzyme Supplementation on Gut pH in Broilers

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Target Audience: poultry producers, feed millers and poultry nutritionist.

***Abstract* – This study was carried out to determine the effect of diet type and enzyme supplementation on the pH of three sections – crop, ileum and caecum - in broiler GIT (gastrointestinal tract). A total of one hundred and fifty (150) ANAK 2000 broiler chicks were purchased, brooded for seven days and randomly distributed to three dietary treatments having five replicates of ten birds per replicate. The control diet was maize – soy bean meal based having 550gm/kg of maize of which 200gm was replaced with wheat offal in the other two dietary treatments. One treatment was supplemented with enzyme (Roxazyme G 2G ®) at an inclusion rate of 200gm/tone of complete feed and the other was not supplemented with enzyme. The experiment was designed as a complete randomized design and lasted thirty five days.**

On day 35, two birds per replicate were slaughtered and pH of the contents of the crop, ileum and caecum were determined. Determined pH values were subjected to linear contrast analysis using SPSS package volume 17 and significant means separated by Duncans Multiple Range test.

Diet type significantly ($p<0.01$) reduced gut pH in crop and not in the ileum and caecum. Crop pH was numerically lower in enzyme supplemented (5.048) diet containing 200gm wheat offal than the diet without enzyme (5.274). Crop pH was more acidic in 200gm wheat offal diet than the control diet (6.122). Ileum pH across the treatments was mildly acidic ranging from 6.356 in the control diet, 6.424 in wheat offal with enzyme and 6.044 in wheat offal without enzyme diet. The pH of the caecum was almost neutral across all treatments.

Diet type lowers crop pH which enables proliferation of *Lactobacillus*, while enzyme supplementation may result in providing nutrients for beneficial bacteria to grow and multiply at the expense of pathogenic bacteria.

***Keywords* – Broilers, Diet–Type, Enzyme Supplementation, Gut pH.**

I. Introduction

Essentially, enzyme supplemented poultry diets results in enhanced growth, better/ improved feed conversion and flexibility in least cost formulation of feed. Although poultry eat to satisfy their requirement for energy; however, the effect of the diet on certain gut factors cannot be overlooked. Flexibility in least-cost formulations which allows for inclusion of agro industrial by-products in poultry diets may adduce certain benefits to the birds which may not be obvious. This brings to the fore the additional benefits of stabilization of gut microflora as previously published [1] and consequently improved well being of the bird [2], which cannot be over emphasized. Such factors as feed pH, acid binding capacity (amount of acid required to lower the pH of a solution), the duration between feeding and pH measurement, type of microbial population present, digestive secretions and mechanical actions of the digestive tract can affect pH of the gut. Digesta pH is one of the major gastrointestinal environment influences on nutrient bioavailability [3]. As a result, accurate determination of digesta pH in broilers could act as a tool to indicate potential for optimum gut health and hence maximize nutrient absorption.

The gut microflora type and population can be affected by the type of diet [1] and this can ultimately affect nutrient digestion and absorption. Once nutrient digestion and absorption has been affected then overall performance of the bird will also be affected. Broiler birds need enzyme supplemented diets to break down the NSP (non – starch polysaccharides) present in agro-industrial by-products when included in their diet. *Lactobacillus* proliferates better in an acidic environment and is resident more in the crop of broilers which has an acidic environment suitable for their growth. An increase in crop pH would signify a lowered colony forming unit (CFU) of *Lactobacillus* and increased CFU of pathogenic bacteria. As digesta chyme passes into the gizzard and latter small intestine it is of benefit to the bird when the crop pH is within its acceptable range. As some of the resident *Lactobacillus* pass into the ileum, they exert a stabilizing effect on the microflora population in the small intestine. The type of diet either fibrous or not and supplementation with an enzyme may ultimately affect the above mentioned factors via the pH of the various sections of the gut. The methodology of pH determination previously measured [4] in the gut may affect the accuracy of pH measures. As a result of this, this experiment was carried out to determine the effect of diet type and enzyme supplementation on gut pH of various sections of the gut in broilers. The methodology for pH determination was improved upon as opposed to the initial method used [2].

II. Materials and Method

This research was carried out at the poultry unit of Niger Delta University Teaching and Research farm. Roxazyme G 2G, a non starch polysaccharide (NSP) degrading enzyme was supplemented to one of the treatment diets. It is an odorless granulates which is soluble in water. It contains an enzyme complex derived from *Trichoderma*

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longibrachiatum. It has an effective pH range of 3.5 - 5.5 and a temperature range of 30 - 55°C. The dosage range was 200gm per ton of complete feed. A hundred and fifty day old unsexed broiler chicks (ANAK 2000) were purchased from Elohim Farm in Yenagoa, Bayelsa state. The chicks were brooded for a week. The chicks were weighed and randomly distributed to three treatments having five replicates and ten birds per replicate at the end of the brooding period. Distribution of birds to replicates was done without regards to sex. The duration of the experiment was 35days. Feed and water was supplied *ad libitum*.

Three experimental diets were formulated. The first diet which served as control was maize – soybean meal based having 550gm/k of maize. The other two treatment diets had 200gm of maize replaced with wheat offal. One was not supplemented with enzyme – treatment 2 and the other was supplemented with enzyme (Roxazyme G 2G ®) – treatment 3.

On day 35, two birds per replicate were slaughtered from each treatment for determination of gut pH in different sections of the GIT. The pH of the crop, ileum and caecum were determined. The ileum was defined as 2cm posterior to merkels diverticulum and 2cm anterior to the ileal – caecal – colonic junction. A pH (HANNA instrument Hi9024 micro computer pH meter) meter probe was inserted directly into the crop content until a stable value was recorded digitally as the crop pH. Due to the size of the ileum and difficulty in inserting the probe directly into the ileum without any incisions, the ileum content was emptied into a plastic sterile sample container and the probe used to stir the ileal content for one minute to obtain a stable value digitally. A similar procedure was applied for the caecum. The pH values were read on the farm. The pH meter was a digital battery fitted type which made on farm pH determination easier.

A hundred gram each of experimental diet was collected and set aside for proximate analysis. Proximate analysis of experimental diets was carried out according to [5]. Gross and nutrient composition of the experimental diets is as indicated in Table 1. The experiment was arranged as a complete randomized design. Data collected on pH of gut sections were subjected to linear contrast analysis in SPSS 17 and significant means separated with Duncan's Multiple Range test [6].

III. Results and Discussion

It is well known that the nature of the diet can influence the processes of digestion and absorption, and that the conditions in the digestive tract and levels of microbes such as *Lactobacillus* change after feeding. Feed pH and acid binding capacity can vary depending upon feed ingredients and would therefore influence the effectiveness of acidifiers and buffers produced by the gastro intestinal tract. Proximate composition of the experimental diets (Table 1) did not show any large disparity in protein content of the diets, dry matter concentration or any other nutrient analyzed.

i. Crop pH

The most accurate representation of broiler gastrointestinal pH is to insert a pH probe directly into the gut lumen [4]. The findings of the current study suggest that pH of the crop was significantly ($p<0.01$) reduced by diet type and numerically lowered by enzyme supplementation (Table 2). This result is considered most accurate as the pH meter probe was directly inserted into the crop content compared to previous reports [1]-[2]. The authors added water to the digesta before determination of pH which appears to dilute hydrogen ion concentration, causing pH to rise and may not have given a true representation of the pH of the gut sections examined [4]. Bacterial populations found in the crop are generally composed of acid – producing bacteria that reduce the pH of the crop contents [7]. Research findings have also reported crop pH to be as low as 5.5 [8] and 5.0 [9] in 42 days old broilers. This further supports results obtained in the current study. Values recorded were 5.274 in WO diet without enzyme and 5.048 in WO with enzyme diet respectively. These findings suggest that diet type (wheat offal) with or without enzyme supplementation favored the growth of lactobacillus present in the crop. Two reasons may also be responsible for the observed differences in pH. Firstly, it has been well documented that *Lactobacillus* secretes organic compounds (mostly lactic acid) which tend to reduce the pH of the digesta. Secondly, hydrolysis of insoluble NSP present in wheat offal may further release intermediate products such as fructo – oligosaccharides and xylo – oligosaccharides. Digestion of these low molecular weight oligosaccharides will generally result in an increase in the number of *Lactobacilli* and *Bifidobacterium* with a consequent decrease in *Clostridia* and *Enterobacterium* as reported previously [10]. The Roxazyme G2G added to the feed is mostly active over acidic pH range of 3.5 - 5.5. The crop pH provided a suitable condition for enzyme activity. Hence, the findings of this study support the notion that *Lactobacillus* countered pathogenic organisms by creating acidic conditions that inhibit growth and proliferation of competing pathogens.

ii. Ileum pH

Digesta ph is one of the major gastrointestinal environment influences on nutrient bioavailability [3]. An accurate determination of digesta pH in broilers could act as a tool to indicate potential for optimum gut health and hence maximize nutrient absorption. Compared to previous findings [11]-[1] ileum pH recorded in the current study were more acidic. It is supposed that although the pH probe could not enter the ileum and the ileum pH had to be determined outside it, however these results are more likely closer to the actual pH value that would have been obtained if the probe had been inserted directly into the ileum. This is as a result of difference in methodology of pH determination. The process of removing the digesta and stirring it outside appeared to have negatively affected the accuracy of the reading [4]. Diet type and enzyme supplementation did not significantly influence pH in the ileum. The least pH value was recorded in broilers fed WO diet without enzyme supplementation. According to previous findings [8],

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duodenal pH was 5.8, jejuna, 6.6 and ileum 7.5 respectively at 42 days of age in broilers. These values differ from that recorded in the current study (Table 2). A value of 6.356 was recorded in the control, 6.044 and 6.434 in WO diet without enzyme and with enzyme supplementation respectively on day 35 which was actually day 42 and age of the birds. The relationship between pH and age in the current study suggests that as the digestive system matures the relationship between gastric acid production and pancreatic bicarbonate secretions change. The subsequent increase in the pH of the small intestine suggests a maturation of the digestive tract with pancreatic bicarbonate being secreted at an appropriate level to compensate for the low pH of the intestinal contents entering the duodenum prior to entering the ileum.

iii. Caecum pH

Unlike the crop, microbial populations in the caecum are mostly *Eubacterium*, *Clostrodium*, *Fusobacterium* and *Bacteroids*. As mentioned before, even though the pH value recorded for this section of the gut was slightly acidic (Table 2), the short fall in methodology used for its determination could have been a factor. Generally speaking, pH values in the current study although not significantly different across the treatments were more acidic compared to values recorded in a previous study [1]. In spite of the limitation of the methodology used in that study, the authors reported diet type and enzyme supplementation significantly ($p<0.05$) reduced pH of the caecum.

VII. Conclusion and Application

From the findings of the current study, it can be concluded that;

1. Diet type lowers pH of the crop which can provide a suitable environment for growth and proliferation of *Lactobacillus* a beneficial microbe.
2. Enzyme supplementation can induce a release of low molecular weight polysaccharides that can serve as prebiotic for beneficial bacteria.
3. Accurate determination of digesta pH in broilers could act as a tool to indicate potential for optimum gut health and hence maximum nutrient absorption.
4. A spear tip piercing pH probe will be the best means of determining gut pH without altering the values obtained.
5. Exposure of the gut digesta to air by removing it from the gut prior to pH determination negatively affects the accuracy of pH reading.

VIII. Acknowledgement

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Ruth Tariebi Seimiekumo Abule (nee OFONGO) is a specialist in poultry nutrition and animal biotechnology. She lectures in the department of Animal Science, Niger Delta University. Dr Abule has a Ph.D degree in Animal Nutrition from the University of Ibadan. She was a DAAD scholar (Visiting research student) at the Institute of Animal science University of Bonn Germany, her research is focused on Effect of nutrition on gut health and integrity in poultry. The role of nutrition on gut microflora and gut related disease in poultry. Genetic characterization of gut microflora in monogastric and ruminant animals using molecular techniques. Physiological function of the gut in relation to colonization ability of gut microflora. Influence of feed additives on health and performance of monogastric animals under normal and disease challenge condition. She has published several peer reviewed articles and has attended few international and local conferences where she has presented papers.

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Table 1. Gross and nutrient composition of experimental diets

Ingredients	M- SBM	200gm WO - enzyme	200gm WO + enzyme
Maize	550	350	350
Soybean meal	330	330	330
Fish meal	40	40	40
Cassava starch	42	42	42
Wheat offal	0	200	200
*Constant ingredients	38	38	38
Total (1000gm)	1000	1000	1000
M.E. (Kcal/kgDM)	3024	3036.03	3036.03
C.P gm/kgDM	214.94	218	218
Analyzed composition			
Dry matter (gm)	739.5	693.5	698.5
Ash (gm/kgDM)	154.2	194.7	142.3
Crude protein	238	223	248
Ether extract	58.1	49.0	54.4
Crude fibre	64.9	71.9	64.2

***:** mineral vitamin premix (2.5gm), DL Methionine (1.5gm), bone meal (21gm), oyster shell (10gm) salt (3gm). M.E.: metabolisable energy, C.P.: crude protein, M: maize, SBM: soybean meal, WO: wheat offal

Table 2. Effect of diet type and enzyme supplementation on gut pH in broilers

Gut sections	M - SBM	200gm WO - enzyme	200gm WO + enzyme	SEM	*P value*
Crop	6.122^{b}	5.274^{a}	5.048^{a}	0.012	0.004
Ileum	6.356	6.044	6.434	0.009	0.208
Caecum	6.660	6.760	6.746	0.009	0.886

ab: means along the same row with different superscripts are significantly different ($p<0.01$). M: maize, SBM: soybean meal, WO: wheat offal

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